

Methods for Detecting Teratogenic Agents in Man*

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At a multidiscipline international meeting sponsored by L'Institut de la Vie held at Guadeloupe in January 1974, current methods for detecting teratogenic agents were outlined and discussed. Recommendations of the participants of the conference were: recognize the limitations of the present defenses against teratogenic agents; educate the public and medical profession about the known human teratogenic agents; select for animal teratogenicity screening among new and existing agents by emphasizing substances to which the entire population will be exposed, agents to which pregnant women are exposed, viruses which are found to persist in the human fetus, and agents which have become suspect from clinical experience; recognize that nearly all compounds have a fetotoxic dose but that this does not imply teratogenicity; encourage the development of new, quick *in vitro* testing methods for detecting teratogenic agents; monitor for sudden increases in the frequency of specific malformations in newborn infants and in aborted fetuses; assure that expert multidiscipline committees are available to evaluate the threat when suspected teratogens are reported; improve teratology information storage and retrieval systems by record linkage of clinical data, linkage between computer systems, and universal identifier system for chemical compounds and congenital malformations; foster the exchange of data, particularly those held by the pharmaceutical industry.

Introduction

The question "Can we prevent another thalidomide disaster?" would have to be answered in the negative today, 14 years after the fact. Although recurrence today is much less likely, the possibility may never be eliminated. The purposes of the present paper are to outline and discuss our current methods for detecting teratogenic agents and to make some wide-based proposals for implementing new approaches to the problem.

A teratogenic agent is a drug, chemical, virus, or physical agent which by acting during the embryonic or fetal period alters morphology or subsequent function in the post natal period. Although

over 600 of these agents have been cataloged in the experimental animal, hardly more than 20 of this group are known (1-4) to be active in man (Table 1).

Errors of intrauterine development are estimated to produce a prenatal loss in the human in excess of 40%. Serious defects can be detected in about 3% of newborn babies, and the number detected gradually rises to about 8% during the following 10 years. Professor Maurice Marois, Director of l'Institut de la Vie, has recognized the impact of this problem on the quality of human life and has initiated efforts toward evaluating the current status of our teratologic defenses and integrating and improving our emerging and existing methods. This was translated into a multidiscipline international meeting held on the island of Guadeloupe in January of 1974. The participants of this conference believed at the close of the meeting that some important understandings and conclusions had been reached and that these should be recorded in a form that would be accessible to interested scientists in universities, governments, and

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Table 1. Known teratogenic agents in man.

Radiation
Therapeutic
Radioiodine
Atomic weapons
Infections
Rubella virus
Cytomegalovirus
Herpes virus hominis I and ?II
Toxoplasmosis
Syphilis
?Varicella virus
Venezuelan equine encephalitis virus
Maternal metabolic imbalance
Endemic cretinism
?Diabetes
Phenylketonuria
Virulizing tumors
Alcoholism
Drugs and environmental chemicals
Androgenic hormones
Aminopterin and methylaminopterin
Cyclophosphamide
Busulfan
Thalidomide
Mercury
Chlorobiphenyls
Diethylstilbestrol
Diphenylhydantoin
Coumarin derivatives

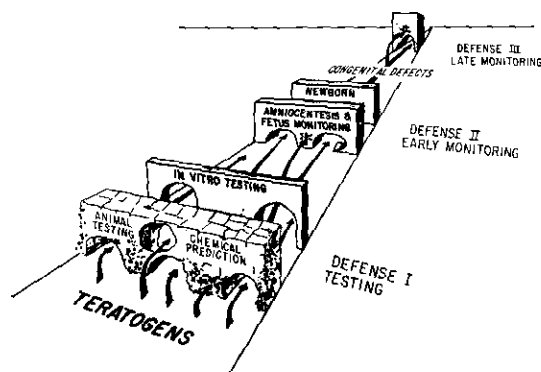


FIGURE 1. Perspective of our major defenses against teratogenic agents. The first defense is standard testing carried out in pregnant animals. A limited knowledge of the biologic activity of chemical structure contributes. The second wall, which should be in close association with the first, consists of *in vitro* testing by tissue and organ cultures as well as studies of ova and whole embryo explants. Defense II, or early monitoring of fetus and newborn, is necessary because the first defenses still are inadequate. Late monitoring is illustrated by the appearance of vaginal carcinomas in young women exposed in utero to diethylstilbestrol (6). This later defense is manned by the alert medical practitioner. Figure reproduced with permission from Shepard (2).

industries. In recording the consensus of our deliberations on such a very wide ranging subject, we are bound to strike some point of disagreement in the mind of every discerning reader. A more complete report of the proceedings including discussions, appears elsewhere (5).

It is possible to conceptualize existing defenses as three walls or hurdles (Fig. 1). In addition to existing potential teratogenic agents, an estimated 2000 new chemicals are introduced into the environment each year. The standard teratogenicity testing of chemicals in pregnant laboratory animals would represent the first defense wall. This defense, erected only after the devastating effects of thalidomide, is held by a few to be effective, but a majority of scientists recognize that it has severe limitations. The use of chemical structure as a predictor of teratogenicity is theoretically of great promise but to date has seen little practical application. A number of new tests based on *in vitro* techniques are available but are relatively little used at present. The utility of these methods, which include tissue and organ culture, ova culture, and whole embryo culture, will be discussed at some length below. A second defense (or more properly a watchtower) consists of monitoring and is necessary because of the admitted inade-

quacy of the first defense. Monitoring may be carried out on embryonic and fetal loss, fluid obtained from the amniotic cavity, or on the newborn. A third defense, which is only beginning to be used, is that of late monitoring, an example of which might be the demonstration that women who were exposed to diethylstilbestrol during the first trimester of fetal life are at increased risk for vaginal cancer (6).

Animal Testing and Pharmacologic Prediction

Unfortunately, the narrow view of teratology often includes only the administration of various drugs and chemicals to small laboratory animals. Although this area is a substantial and legitimate area of teratology, it is clear that teratologists include a very wide spectrum of scientists encompassing many endeavors from cell biology to human epidemiology.

What priorities should guide us in a choice of agents to be tested in pregnant laboratory animals? Substances to which the entire population will be exposed should receive priority. Examples of this are the food additives (GRAS list, generally

released additive substances) currently under scrutiny by the Federal Drug Agency, and compounds such as nitrilotriacetic acid (7). Nitrilotriacetic acid, a biodegradable substitute for phosphates in detergents, if widely marketed, might be ingested by humans at a level of around 100 $\mu\text{g/day}$. A second category of agents to be tested are those to which pregnant women may be exposed. Some substances might inadvertently be ingested by a pregnant woman, and others such as antibiotics and diuretics may be necessary therapeutic agents. A third category are the virologic agents. If it can be shown that a virus resides in the embryo-fetus, careful and long-term testing is indicated. Inadvertent *in utero* exposure to live vaccines or virus diseases before therapeutic or spontaneous abortion should merit virologic culture of conception products on an ongoing basis. Finally, any agent which becomes suspect from clinical observations certainly deserves immediate attention.

The question of the species to be used and the number of test animals was discussed. Some participants recommended the testing of a mongrelized or genetically heterogeneous groups of animals in order to identify any genetic subgroup which might be highly susceptible to a teratogenic agent. Although this is an important goal, the field of pharmacogenetics is probably not sufficiently technically developed to make this feasible in the near future. The use of subhuman primates should probably be reserved for special situations where an agent must be used during human pregnancy or where there is clinical reason to suspect human teratogenicity.

The dosage of the test substance should be maximal but should not interfere appreciably with the health of the maternal animal. Practically any substance in high enough dosage can be shown to be fetotoxic. Wilson and many others recommend that a fetotoxic response should be obtained when testing substances. The general acceptance by regulatory agents that compounds do have a fetotoxic dose should help to prevent us from excluding the many therapeutic agents which are unnecessarily discarded because of fetotoxic effects at high dose. We agree with the report by the Canadian Ministry of Health and Welfare (8) that high dose pulses of the test substance should be given for relatively short periods during organogenesis. This tends to decrease maternal adaption to prolonged exposure which might lead to reduced levels reaching the fetus. Because of the findings that the fetus may be more susceptible to carcinogens than the postnatal animal (9), more emphasis should be

placed on the appearance of tumors in animals tested over several generations.

At some point in the future our knowledge of pharmacologic action of chemicals should allow us to predict accurately the biologic activity of new substances. This field of endeavor should be encouraged in every way, but at the present time it offers little aid in preventing human teratogenicity.

In Vitro Testing

Many participants of the conference accepted the idea that *in vitro* testing should play a more important role in our defenses against teratogenic agents. However, it was also accepted that routine screening by most of these methods would be of little help and would certainly not displace the use of pregnant animal testing. The use of the *in vitro* tests is of a special importance for elucidating mechanisms of teratogenesis. With better understanding of the mechanisms, more intelligent measures can be directed toward interruption of human embryopathy. A number of investigators recommended that human material, particularly that received during therapeutic abortion, be used for tissue in organ culture testing of teratogenic agents. This material should be utilized by developmental biologists after careful peer review, and with fully informed consent of the donors.

Human Monitoring

The 3% of neonates with congenital defects represent only the tip of a larger iceberg of embryonic and fetal loss. We can learn a great deal by studying the larger part of this iceberg. One might expect that prenatal losses would be a more dramatic and sensitive index of the response to a teratogenic agent. By study of embryos and fetuses from spontaneous abortions in the first trimester, an earlier warning might be established which could provide epidemiologic information some 6 months before a teratogenic effect would be detected in newborns. Another advantage to this early monitoring is a shortening of the period from the time of teratogenic exposure to the time of inquiry of the mother. This could be less than a week as compared to the 7-8 month interval when the history is taken from the mother of a neonate. Systematic monitoring of human embryonic and fetal loss should be introduced.

Monitoring facilities for defects in neonates exist in many countries. These would probably fail to detect minor changes in brain function or long term carcinogenesis. Generally only very easily recognized physical defects are recorded; the larger portion of congenital disease identified after the newborn period (60%) is usually not included. Although these systems involve many variations due to artifacts associated with data collection, a continuous recording by time and place, and registry of congenital defects should provide an important warning of teratogenic action by new chemical, physical or infectious agents. The neonate data may be used to test hypotheses, as illustrated by the initial report from Australia (10) that a tricyclic antidepressant (Imipramine) might be the cause of limb reduction defects. Using their own case data, the monitoring groups in Atlanta and Canada were able to make a rapid, detailed survey of all women having given birth to children with limb defects. Their negative results (11, 12) in hundreds of women offered strong evidence that this drug was not a highly potent human teratogen.

Another concept of importance is that of record linkage (birth, death, hospital, pharmacy, and school records). Some of the epidemiologic information required may involve two- or three-generation studies. This factor tends to exclude observation by one observer, but computerized methods for linking records can overcome this problem to some degree. With the present day computer systems it should be possible to link birth records of children with malformations to drug prescriptions given the mother, previous disease, and ill health of the parent and other affected members. A recent publication from the Kaiser Permanente System (13) reports the use of record linkage to study the association between prescriptions filled for women and serious congenital defects in their offspring. A fourfold increase in defect rate was found when the mothers took the commonly used tranquilizers, meprobamate and chlorthalidone during the first 42 days of gestation. The rate was not similarly increased in a group taking barbiturates. Significantly, all the prescriptions were written by physicians not involved in caring for the pregnancy. Those concerned with the organization of health care systems should be aware of the need for designing methods of record keeping in a form suitable for record linkage.

Regulatory agencies should set up standard bodies of experts to investigate any suspected teratogens. These groups should consist of pediatricians, obstetricians, epidemiologists, and other ap-

propriate consultants. In general, the observers reporting the suspected teratogen should not be responsible for organizing or conducting the full investigation.

Many teratologists assume that a large variety of congenital defects result from several development-controlling genes acting in concert with environmental agents. Fraser and his students have illustrated this mechanism in certain inbred strains of mice (A/J) and are beginning to make practical human applications to the control of certain defects (14, 15). The A/J mouse exhibits a higher natural incidence of cleft lip and palate than certain other strains, and this is related to differences in the topographic relations of the embryonic facial processes (16). Other controlling genes for the development of the palate are known to predispose to clefts (14). Examples such as shortening of the head, changes in mandibular length, or mechanisms that by causing tongue obstruction might prevent normal palatal shelf closure have been studied by using mutant genes or inbred animal models. Most of the environmental agents (aspirin, cortisone) known to produce clefts in animals are more effective in these inbred strains. It seems imperative to accelerate the application of these principles to man. This requires quantitative methods for identifying the susceptible human genotypes (the A/J man?) and the environmental agents that might contribute to the multifactorially caused defects. Since the modification of gene action may be technically impossible for some time to come, the removal of environmental agents may be the simplest solution to this problem. The frequency of malformations appears to be about doubled, and the frequency of cleft lip is increased about 20-fold in the offspring of mothers being treated for chronic seizure disorders with diphenylhydantoin or trimethadione (17, 18). Is this an effect of the drug or is it possible that these women have a genotype that predisposes to both seizure disorder and clefts?

The use of amniocentesis to detect genetic disease or neural tube closure defects in high risk populations is assuming an important role, and there is an increasing amount of practical genetic information which should and is being given to parents who wish to participate actively in preconceptual or prenatal planning for their offspring.

Another example of the importance of identifying genotypes may be made by workers in the field of pharmacogenetics. Certainly there must exist particular groups of women who because of molecular changes in detoxifying enzymes are more vulnerable to teratogenic action of certain drugs.

The identification and description of new congenital defect syndromes is of clear importance if we are to detect teratogenic agents. An example of this is the association of maternal anticoagulant therapy (warfarin) with a rare dyschondroplasia, Conradi Syndrome (19). Another good example is the identification by Jones et al. (20) of a recognizable fetal alcohol syndrome.

Data Storage and Retrieval

As indicated by Dr. Sune Larsson, between 1957 and 1972 there has been a doubling of the number of scientific articles on developmental subjects with about 250,000 appearing in 1972. The description of new dysmorphology syndromes in the human is creating a staggering task in the efficient application of science to medical care. There is a need for certain types of texts which can be produced from computer tape print-outs making the material available to a computer user. Examples of such books are McKusick's *Catalog of Mendelian Inheritance in Man* (21) and Shepard's *Catalog of Teratogenic Agents* (1). These books have the advantage of rapid, easy revision and cheap production.

Other data bank systems exist in various government and corporation offices, but many of the data banks cannot be combined due to technical and/or political problems. An important element for the integrated use of these data banks is a universal identifier system with each chemical compound being assigned a separate number, such as the Chemical Abstract Service (CAS) number. The political problems are in part associated with the job insecurity created by one computer system cannibalizing another. An answer to this is to create a symbiotic system (umbilical cord) which allows both merging systems to draw on each other's data. Such a system is planned under the title of International Registry of Potential Toxic Chemicals, sponsored by the United Nations Environmental Programme (UNEP). In the case of teratology, there is some possibility that a large amount of useful information, especially on positive findings, is buried and unpublished in the files of the pharmaceutical industry. Steps should be taken on an international basis to foster the collection of these data through the agency of existing organizations such as the Teratology Information Center (TIC) in Stockholm. Perhaps if the pharmaceutical companies were to list with TIC their storage of data on certain compounds, the problem of transfer

of large amounts of information could be obviated. At the same time, this would leave the release on request of the information up to the individual pharmaceutical industry. Scientists in the pharmaceutical industry are known to be responsible investigators, and we believe they should be encouraged in every way to contribute to the general task of teratogen detection.

Recommendations

Some recommendations of a group of participants of this conference are as follows.

1. Recognize the limitations of the present defenses against teratogenic agents.
2. Educate the public (and medical profession) about the known human teratogenic agents (Table 1).
3. Select for animal teratogenicity screening among the vast number of new and existing agents by emphasizing: substances to which the entire population will be exposed, agents to which a pregnant woman is exposed, viruses which are found to persist in the human fetus, and agents which become suspect from clinical observations.
4. Recognize that nearly all compounds have a fetotoxic dose but this does not imply that they are teratogenic.
5. Encourage the development of new quick *in vitro* testing methods for detecting teratogenic agents and especially for determining the mechanism of action of those that are known.
6. Monitor for sudden increases in the frequency of specific malformations in newborn infants and in aborted fetuses. Where suspected teratogens are reported assure that there are expert multidisciplinary committees to evaluate the threat.
7. Improve our teratology information storage and retrieval systems by: record linkage of clinical data, linkage between computer systems, universal identifier system for chemical compounds and congenital malformations, and fostering exchange of data, particularly those held by the pharmaceutical industry.

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